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Finding a Link between the Hormones of the Somatotropic Axis and Growth Rate of Juvenile Steller Sea Lions

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INTRODUCTION

The changes in the somatotropic axis [growth hormone (GH), insulin-like growth factor (IGF)-1, and IGF binding proteins (IGFBP)-2, and -3] that occur throughout development have been well-documented in domestic animals (Brier et al., 1999, Govoni et al., 2003). Furthermore, these developmental changes have also begun to be documented in marine mammals (Richmond et al., 2010a, 2010b). However, the changes in the components of the somatotropic axis during the development of the Steller sea lion (*Eumatopias jubatus*) have not yet been examined. Since the hormones of the somatotropic axis correspond predictably to changes in growth rate in domestic animals, it may be useful to use these hormones as an indicator of growth rate in the Steller sea lion.

The Steller sea lion, found throughout the North Pacific rim, has experienced an 80% population decline since 1970 (Loughlin et al., 1992). Most recently, the decline has been exclusively observed in the genetically distinct Western population, whereas the Eastern population has been stable or increasing in numbers (Hoffman et al., 2006; Pendleton et al., 2006). The cause for the decline of the Western population is unknown, although many hypotheses have been suggested (Trites et al., 2007; Atkinson et al., 2008). The most prominent hypothesis suggests that reduced prey availability, resulting from either a change in environment, commercial fishing activities, or a combination of the two, has led to a change in prey quantity and quality in certain geographical areas (Trites et al., 2007; Atkinson et al., 2008). As a result of reduced nutrient availability, the animals that depend on these prey source may experience a decreased nutritional status

(Atkinson et al., 2008). Changes in prey resources may have led to reduced juvenile survival, which is a likely cause for the decline in the total population (York, 1994).

Juvenile Steller sea lions, like other young, developing mammals, must maintain an increased growth rate to survive and attain mature size at an appropriate age. Their immature physiological development (Richmond et al., 2005, 2006) and lack of foraging experience (Pitcher et al., 2005) may put them at a disadvantage compared with adult animals (Horning and Trillmich, 1999). In times of poor prey availability, this may lead to decreased growth (Richmond et al., 2010b). Furthermore, there is a link between growth rate and survival of juvenile pinnipeds indicating that larger juveniles with greater growth rate have greater chance of survival (Muelbert et al., 2003). Understanding the physiological factors that influence growth rate may lead to a new understanding of juvenile survival.

The somatotropic axis is a system of hormones that work in concert to regulate normal growth and development. These hormones are essential to growth, development and regulation of metabolism (Breier, 1999). The concentrations of these hormones vary predictably depending on the age and nutritional status of the animal (Govoni et al., 2003; Richmond et al., 2010a).

In domestic species, the somatotropic axis can be used to link growth physiology, development, and nutritional status (Breier, 1999). In many young domestic mammalian species, during the period of rapid body growth, serum concentrations of components of the somatotropic axis have defined patterns of change (Govoni et al., 2003). Studies examining the somatotropic axis in pinnipeds have shown that the hormones have similar secretory patterns relating to age and nutritional status as domestic species (Richmond et

al., 2010a). Therefore, examining the relationship between growth rate and the components of the somatotropic axis that regulate tissue specific growth and nutrient allocation may provide insight into the survival of juvenile Steller sea lions to adulthood.

This study aims to determine if GH, IGF-1, IGFBP-2 and -3 change with age and can be used as predictive indicators of growth rate in free-ranging juvenile Steller sea lions.

LITERATURE REVIEW

Introduction

The Steller sea lion, *Eumetopias jubatus*, found throughout the North Pacific rim, has experienced an 80% population decline since 1970 (Loughlin et al., 1992). Changes in prey resources may have led to reduced juvenile survival, which is a likely cause for the decline in the total population (York, 1994). In times of poor prey availability, juveniles are at a disadvantage due to their immature physiology and lack of foraging experience, which may lead to decreased growth (Richmond et al., 2010b). Furthermore, there is a link between growth rate and survival of juvenile pinnipeds indicating that larger juveniles with greater growth rate have greater chance of survival (Muelbert et al., 2003).

In domestic species, the somatotropic axis can be used to link growth physiology, development, and nutritional status (Breier, 1999). In many young domestic mammalian species, during the period of rapid body growth, serum concentrations of components of the somatotropic axis have defined patterns of change (Govoni et al., 2003). The focus of this literature review will be on Steller sea lion life history, the decline of the Steller sea lion population, the hormones of the somatotropic axis, their developmental changes and the influence of nutrition on the serum concentrations of GH, IGF-1, and IGFBP-2, -3.

Steller Sea Lion Life History

Steller sea lions are classified as Otariids. This group of animals nurses their young for longer periods of time and has less energy-dense milk than phocids, or true seals. Therefore, Otariids grow at a slower rate than phocids (Burns et al., 2004). The

largest species of Otariid, Steller sea lions, exhibit sexual dimorphism with males weighing 1000 kg and females 250 kg (Winship et al., 2001). During the breeding season, these animals gather on beaches and outcroppings, called rookeries, in large harems of multiple females and a dominant male (Perrin et al., 2002). Individual adults will return to the same rookery every year to breed (Raum-Suryan et al., 2002). Steller sea lion pups are born in mid-June and remain with cows that nurse the pup for the first 1 to 2 weeks of life (Pitcher et al., 2001). After this time, the female begins nightly foraging trips leaving the pups alone (Pitcher et al., 2001). However, the pups continue to nurse throughout their first year of life and it is not uncommon to see a 2- or 3-year old nursing (Pitcher et al., 2001). Occasionally, females will nurse a yearling pup along with a new pup of the year (Manisalco and Parker, 2009).

Pups begin eating solid food (fish) around 5 months of age, but the precise age of nutritional independence is unknown (Raum-Suryan et al., 2002). Pups first enter the water 2 to 4 weeks after birth and begin to disperse with their mothers from their natal rookeries around 3 months of age (Raum-Suryan et al., 2002). As juveniles, these animals disperse widely from their natal rookeries, but upon reaching sexual maturity they remain close to the beaches on which they were born (Raum-Suryan et al., 2002).

The sexual dimorphism in this species can be seen throughout growth from 1 year to maturity (Winship et al., 2001). Young females grow at a slower rate than males, attaining a mature size at 4 years of age (Winship et al., 2001). Females are sexually mature around 3 to 8 years of age and will continue to breed until 20 and 30 years of age (Perrin et al., 2002). In contrast, males show a maximum growth rate around 4 years of age, which declines until reaching mature size at around 9 years of age (Winship et al.,

2001). Males sexually mature at the same age as females, but are not large enough to defend a harem of females until approximately 9 years of age (Perrin et al., 2002). Winship et al. (2001) suggested that rapidly growing juvenile Steller sea lions may survive better than slowly growing individuals of the same age. Furthermore, Muelbert et al. (2003) had similar findings which indicated a similar relationship between growth rate and survival in young pinnipeds. In this study, larger pups with greater growth rates had an increased probability of survival (Muelbert et al., 2003).

Overall, Steller sea lions are a slow growing species compared with other pinnipeds (Burns et al., 2004). Moreover, larger, more rapidly growing individuals are better adapted for survival than their slower growing counterparts. This indicates that larger, faster growing juvenile Steller sea lions may have a greater chance of survival.

Two Populations

In Alaska, Steller sea lions can be divided into two genetically distinct populations, a Western population, which includes animals ranging west of Cape Suckling (144 degrees west longitude) and an Eastern population, including those animals ranging east of the same longitudinal mark (Hoffman et al., 2006). Through the investigation of mitochondrial DNA (mtDNA), the two populations cannot be traced back to a single female, but instead are most likely the result of glacial separation (Bickham et al., 1996). In other words, these two populations had to have been separated for an extended period of time to allow for the evolutionary development of these distinct mtDNA genotypes (Bickham et al., 1996). Furthermore, the genotypes found in the Western population are not observed in the Eastern population, suggesting that there is

little gene flow between the populations (Bickham et al., 1996). For example, tracking young individuals has shown that there is little migration between these two populations (Raum-Suryan et al., 2002).

There is a difference in weaning age between these two genetically distinct populations (Pitcher et al., 2004). Young from the Eastern population nurse for a longer period of time than do those from the Western population (Pitcher et al., 2004). This longer period of nursing may give juveniles of the Eastern population a greater survival advantage over those of the Western population by allowing for a greater growth rate during similar ages.

Whether a third division in the species can be made to separate a genetically distinct Asian population of Steller sea lions has been debated in the past (Hoffman et al., 2006). Although there is a defined break between the mtDNA of the Western and Eastern populations, the same is not true of the Asian population (Hoffman et al., 2006). The Asian population is considered a sub-population of the Western Stock, and shows similarities in mtDNA that are not significantly different enough to consider it genetically distinct (Hoffman et al., 2006). The discovery of these two populations has significant implications for the conservation and management of this endangered species.

Population Decline

In 1989, it was estimated that the world population of Steller sea lions was approximately 100,000 (Loughlin et al., 1992), which is a dramatic decrease from the estimated 250,000 animals in 1960 (Kenyon and Rice, 1961). The Western population declined by 85% from 1970 to 2000 and is now classified as Endangered (Pendleton et

al., 2006). Conversely, over the same time period, the Eastern population increased at a rate of approximately 3% per year (Pendleton et al., 2006).

Many hypotheses have been made for the cause of this difference in population dynamics. The main hypotheses for the decline have been divided into anthropogenic causes and environmental causes (Trites et al., 2007, Atkinson et al., 2008). The anthropogenic causes include competition with commercial fisheries, disturbance from research, contaminants from human chemicals, and human hunts, whereas the environmental causes include consequences resulting from global climate and oceanographic changes, which have led to changes in food resources, habitat, predation and possibly disease (Atkinson et al., 2008).

The anthropogenic factors are those that impact the Steller sea lion population directly by causing the death of individual animals or indirectly by causing a disruption in the food supply of these animals. Competition with commercial fisheries has reduced the amount and diversity of prey available to Steller sea lions (Atkinson et al., 2008). This component could result in reduced nutrient intake, or nutritional stress, leading to decreased survival (Atkinson et al., 2008). However, other direct mortality factors, such as incidental by-catch of Steller sea lions during fishing, subsistence hunts, and legal and illegal shooting of Steller sea lions, have been declining in the past decade and are unlikely to have contributed significantly to the decline (Atkinson et al., 2008).

Although there are few data on the effects of pollutants on the health of Steller sea lions, by using data from other pinniped species, it has been inferred that acute toxicity from these chemicals is not the most likely cause for the decline of Steller sea lion populations (Atkinson et al., 2008). Nevertheless, pollutants may be impacting the

recovery of the species (Atkinson et al., 2008). Finally, the impact of research on mortality of Steller sea lions is negligible, having almost no impact on the total population (Atkinson et al., 2008). The most prominent hypothesis is that of nutritional stress, in which a reduced nutrient availability has led to differential survival among age groups (Pendleton et al., 2006).

One of the more prominent environmental hypotheses leading to nutritional stress is that an ocean regimen shift occurred, resulting in change in abundance of Steller sea lion prey species (Trites et al., 2007). Changes in ocean temperatures favor the survival of less energy dense fish, creating a situation in which animals have to find more food to meet their energetic requirements or travel greater distances to find food requiring more energy (Trites et al., 2007). Further, younger, growing animals, specifically newly-independent juvenile Steller sea lions, have a more difficult time obtaining enough food to meet their nutritional requirements (York, 1994; Trites et al., 2007). Thus, these animals are smaller and grow slower, and therefore experience greater mortality resulting in the overall decline of the population (York, 1994; Trites et al., 2007). This "bottom-up process" involves a combination environmental factors bringing about the decline of Steller sea lions (Trites et al., 2007).

Studies have indicated that nutritional stress influences various age groups of Steller sea lions differently (Pendleton et al., 2006). Pendleton et al. (2006) compiled previously recorded population data from the two populations of Steller sea lions and examined the survival of different age groups of animals. York (1994) was the first to propose that the decline of the Western population was a result of decreased juvenile survival. The findings in Pendleton et al. (2006) confirm the hypothesis that juveniles of

the Western population have a decreased rate of survival compared with those of the Eastern population. Interestingly, the survival rate for adult females in the Western population is similar to that of adult males, contrasting with the Eastern population where survival is greater for females than for males (Pendleton et al., 2006). The survival trend in the Eastern population is typical of larger mammalian species, like the Steller sea lion, in which individual males compete for dominance over a large group of females (Pendleton et al., 2006). The similar survival for both sexes in the Western population suggests that the factors responsible for the population decline in the West have disproportionately affected females (Pendleton et al., 2006). These data support the nutritional stress hypothesis (Pendleton et al., 2006). Females have greater energy requirements due to increased energy needs of gestation and lactation (Winship et al., 2002). Therefore, females would be more affected by reduced nutrient availability compared with males (Pendleton et al., 2006).

Through a combination of anthropogenic and environmental factors, the Western population of Steller sea lions has experienced a reduction in prey availability and has thus likely experienced nutritional stress (Trites et al., 2007; Atkinson et al., 2008). Similar data on juveniles are not available; however, if cows and pups are nutritionally stressed it follows that juveniles are also likely to be nutritionally stressed.

Juvenile Disadvantage

Juvenile Steller sea lions, like other developing mammals, must maintain an increased growth rate to attain mature size. As a result, they face greater challenges for survival compared with adults when there is reduced prey availability (Horning and

Trillmich, 1999). Juvenile Steller sea lions are physiologically immature, having a decreased diving capability compared with adult animals (Richmond et al., 2005, 2006). These animals have reduced hematocrit and hemoglobin concentrations along with reduced myoglobin in muscle (Richmond et al., 2005, 2006) all of which is critical for dive capacity of marine mammals. Therefore, these animals are at a disadvantage when foraging compared with older animals, making them more susceptible to shifts in prey availability than adults (Richmond et al., 2005, 2006). Furthermore, juveniles lack the foraging experience possessed by adults (Pitcher et al., 2005). Older juveniles have the ability to dive as deep as adult females (Rehburg and Burns, 2008; Rehburg et al., 2009). Therefore, factors such as learning to locate prey and experience capturing and handling prey are a significant disadvantage to the foraging success and thus the adaptability of young Steller sea lions to change in prey resources is compromised (Pitcher et al., 2005).

In addition to being less capable of finding food in times of decreased prey availability, juveniles are less able to adapt to natural periods of fasting, especially outside of the typical breeding season fast (Rea et al., 2009). Steller sea lions experience periods of fasting as part of their normal life history, and Rea et al. (2009) have shown season has an impact on the capability of these animals to deal with fasting. Younger Steller sea lions lack the physiological adaptations for fasting found in older animals, and this is especially significant depending on season (Rea et al., 2009). Outside of the breeding season, when Steller sea lions typically fast, younger animals are more susceptible to the physiological consequences of starvation and do not switch to protein-sparing metabolism as readily (Rea et al., 2009). As a result, these animals lose greater amounts of lean tissue and have a greater decrease in mass during starvation than their

older counterparts (Rea et al., 2009). Therefore, these animals are at a greater risk of nutritional stress in times of decreased prey availability outside of normal periods of fasting (Rea et al., 2009).

In general, juvenile Steller sea lions face greater challenges in times of decreased prey availability as a result of their immature physiology and lack of experience. Compounding this issue, the energetic requirements of these young, growing juvenile Steller sea lions are greater than those of adult animals (Winship et al., 2002). In other species of pinnipeds, the combination of the increased energy demands and immature physiology leads to decreased growth in juveniles (Horning and Trillmich, 1999). Importantly, there is a link between growth rate and survival of juvenile pinnipeds indicating that larger juveniles with greater growth rates have greater survival (Muelbert et al., 2003). Larger juveniles are better able to cope with fasting (Rea et al., 2009) and better able to dive (Rehburg and Burns, 2008; Rehburg et al., 2009), further aiding mature animal survival (Muelbert et al., 2003).

The Somatotropic Axis

The hormones of the somatotropic axis include GH, IGF-1, and IGFBP (Tuggle and Trenkle, 1996, Breier, 1999). The release of GH from the anterior pituitary gland is controlled by GH releasing factor (GRF) and somatostatin (SRIF), while IGF-1 and IGFBP are produced by the liver and other tissues of the body in response to GH (Tuggle and Trenkle, 1996, Breier, 1999). Their action is essential to growth, development and regulation of metabolism (Breier, 1999).

Growth hormone is a protein, consisting of 190 to 191 amino acids (Secchi and Borromeo, 1997). The secretion of GH is positively affected by GRF, a peptide hormone consisting of 40 to 44 amino acids (Zinn et al., 1994; Tuggle and Trenkle, 1996, Kazmer et al., 2000). Only the first 29 amino acids of this peptide are necessary for, and have biological action in the animal (Kazmer et al., 2000). The hypothalamus secretes GRF in regular pulses (Zinn et al., 1994; Tuggle and Trenkle, 1996, Kazmer et al., 2000). The concentrations of GH are positively correlated with pulses of GRF from the hypothalamus (Rigamonti et al., 2001). The secretion of GH is negatively affected by SRIF, a peptide hormone also released from the hypothalamus (Zinn et al., 1994; Tuggle and Trenkle, 1996, Kazmer et al., 2000). This hormone is synthesized and released as a 61 amino acid precursor peptide, which is further metabolized into active 14 and 28 amino acid peptides (Zinn et al., 1994; Tuggle and Trenkle, 1996, Kazmer et al., 2000). Somatostatin inhibits GRF-induced secretion of GH, but has no effect on baseline GH secretion (Padmanabhan et al., 1995).

Circulating GH in the blood is a key factor in postnatal growth and the regulation of metabolism throughout the life of an animal (Breier, 1999). Produced in response to GH secretion, IGF-1 is a peptide growth factor composed of 70 amino acids (Yun et al., 2005). The structure of IGF-1 is conserved across species (Foyt and Roberts, 1991). This growth factor mediates the anabolic effects of GH, including stimulation of muscle and bone growth (Breier, 1999, LeRoith et al., 2001).

In bone, GH and IGF-1 work in concert to promote growth. Specifically, GH is necessary for the continuation of bone growth as it promotes the production of

prechondrocytes (Isaksson et al., 1987). However, IGF-1 is responsible for stimulating the development of new bone, mediating the action of GH (Isaksson et al., 1987).

In muscle, the effects of GH are mediated via IGF-1 (Connor et al., 2000). The hormone GH may have some direct effects on muscle growth or cell proliferation in muscle (Burton et al., 1994). There are GH receptors on muscle, which stimulate the local release of IGF-1 (Carter-Su et al., 1996). In turn, IGF-1 stimulates protein accumulation, amino acid uptake, protein synthesis, and decreases protein degradation (Connor et al., 2000, Breier et al., 1999). Overall, IGF-1 is responsible for increasing protein accretion (Breier et al., 1999).

In adipose, GH acts directly on adipocytes to influence lipid storage in adipose tissue (Bauman and Vernon, 1992; Mikel et al., 1993). In this way, GH acts to decrease the amount of adipose in the body, stimulating lipolysis and inhibiting lipogenesis, diverting nutrient storage from adipose tissue into storage in muscle or milk (Etherton and Bauman, 1998).

There are six IGFBP, each of which has negative or positive effects on the biological action of IGF-1 (Jones and Clemmons, 1995). The IGFBP vary considerably in size, usually containing between 216 and 289 amino acids (Siwanowicz et al., 2005). The affinity of each IGFBP for IGF-1 and various chemical properties of each IGFBP also vary (Breier, 1999). These proteins share about 80% homology across species (Jones and Clemmons, 1995). Each of these molecules has a different impact on the action of IGF-1 and is under separate endocrine and metabolic controls (Breier, 1999). In the blood, IGF-1 circulates bound to IGFBP changing the biological activity of IGF-1 (Jones and Clemmons, 1995). The IGFBP also regulate the interaction of IGF-1 with its

receptors, further controlling its biological activity (Firth and Baxter, 2002). Furthermore, this transport via IGFBP can extend the half life of IGF-1 in the circulation (Jones and Clemmons, 1995).

Importantly, IGFBP-2 and -3 are most associated with growth rate and are sensitive to nutrient intake (Breier, 1999, Govoni et al., 2003). The protein, IGFBP-3, increases the availability of IGF-1 to bind receptors in target tissues, while IGFBP–2 reduces the availability of IGF-1 (Jones and Clemmons, 1995).

The somatotropic axis consists of multiple hormones with multiple levels of control. In conditions of normal nutrition, these hormones work in concert to promote normal growth and development of the animal by increasing bone and muscle tissues and decreasing fat accumulation. This is achieved primarily through the actions of GH and IGF-1.

Developmental changes in the somatotropic axis

The serum concentrations of the components of the somatotropic axis have defined patterns of change throughout development in mammalian species. There is an age-related decrease in concentrations of GH (Govoni et al., 2003). In cattle, this decrease in GH concentrations is delayed in males compared with females (Govoni et al., 2003). As a consequence, male calves have greater growth rate than females (Govoni et al., 2003). In developing cattle, GH is negatively correlated with growth rate, but only in the first 16 weeks of life (Govoni et al., 2003). However, at any given age, greater GH concentrations are associated with a greater growth rate (Govoni et al., 2003).

In cattle, concentrations of IGF-1 increase with age reaching a plateau around 17 weeks of age (Govoni et al., 2003). Similar to GH, concentrations of IGF-1 differed between males and females, but this difference was not observed until after the plateau in the concentrations of IGF-1 (Govoni et el., 2003). Concentrations of IGF-1 are positively correlated with growth rate (Govoni et al., 2003).

The protein, IGFBP-2, is the most variable of the discussed compounds. It has been debated as to whether this protein decreases or remains the same throughout development (Skaar et al., 1994, Govoni et al., 2002). Govoni et al. (2003) found that earlier in life, IGFBP-2 concentrations increased and then declined until the time of the plateau in IGF-1. The concentrations of IGFBP-2 then remained constant, until becoming highly variable with the onset of puberty (Govoni et al., 2003).

Since IGFBP-3 is the main carrier of IGF-1 in the blood, it mirrors the developmental changes with age observed in IGF-1. In other words, similar to IGF-1, concentrations of IGFBP-3 increase with age (Govoni et al., 2003). Furthermore, the concentrations of IGFBP-3 begin to decrease corresponding with the plateau of IGF-1, and are greater in males than in females (Govoni et al., 2003). As the animals approach puberty, the concentrations of IGFBP-3 become more variable, similar to IGFBP-2 (Govoni et al., 2003).

Most studies examining the somatotropic axis in pinnipeds indicate that the hormones are also correlated with growth (Richmond et al., 2010a, 2010b). In rehabilitated harbor seals, concentrations of IGF-1 and IGFBP-3 are positively correlated with body mass, while GH and IGFBP-2 are negatively correlated with body mass (Richmond et al., 2010a). Similar to domestic species, GH is negatively correlated with

growth rate and IGF-1 is positively correlated with growth rate in pinnipeds (Richmond et al., 2010a). Throughout rehabilitation of young harbor seals, IGF-1 concentrations increase with increased growth rate (Richmond et al., 2010a).

By examining the components of the somatotropic axis that link nutrition, fat metabolism and lean tissue accretion, the impact of decreased nutrient intake on growth rate can be assessed, all of which may have implications for the survival of juvenile Steller sea lions.

The Somatotropic Axis and Nutritional Stress

Understanding the physiological factors that influence growth rate may lead to a new understanding of juvenile survival in Steller sea lions. The somatotropic axis is a bridge between endocrinology, tissue specific growth and nutrient allocation (Govoni et al., 2003, Richmond et al., 2010a, 2010b). Changes in the somatotropic axis can be used to assess the impact of decreased nutrient intake on adipose and lean tissue growth, all of which may have implications for the survival of juvenile Steller sea lions. The concentrations of the hormones of the somatotropic axis vary depending on the age and nutritional status of the animal.

During periods of nutritional stress, GH concentrations in the blood are increased (Breier, 1986). This increase in concentrations of GH is a result of the decreased negative feedback within the somatotropic axis and a decrease in the secretion of inhibitory SRIF (Breier and Sauerwein, 1995). The increase in GH stimulated lipolysis to release stored energy from adipocytes (Kersten, 2001). This energy is essential to maintaining metabolism in a period of nutritional stress. Furthermore, the number of GH

receptors on the liver is decreased (Breier, 1988) and, therefore, there is reduced secretion of IGF-1 in response to GH concentrations in the blood (Breier, 1999). Plasma concentrations of IGF-1 are thus reduced in times of nutritional stress (Breier, 1999). The decrease in IGF-1 prevents nutrients from being utilized in bone and muscle.

Plasma concentrations of IGFBP-2 are increased in a state of under-nutrition as a result of increased transcription rate of the IGFBP-2 gene (Smith et al., 1995; Breier, 1999). This increase in transcription rate has been specifically linked to reduced protein intake (Smith et al., 1995; Breier, 1999). The action of IGFBP-2 in this state restricts the insulin-like action of IGF-1 causing the body to remain in a catalytic state utilizing available energy stores (Jones and Clemmons, 1995; Breier, 1999).

Conversely, plasma concentrations of IGFBP-3 are reduced at times of nutritional stress (Jones and Clemmons, 1995, Breier, 1999). Not only is the actual concentration of IGFBP-3 reduced in the blood, but there is also an increase in protease activity to decrease the affinity of IGFBP-3 for IGF-1 (Breier, 1999). The reduction in concentration of IGFBP-3 further decreases the action of IGF-1.

Severe nutritional stress impacts the hormones of the somatotropic axis in pinnipeds similar to domestic species (Richmond et al., 2010a). Rehabilitated harbor seals that begin the rehabilitation process in a state of nutritional stress or negative energy balance, had increased serum concentrations of GH that decrease over time with re-feeding (Richmond et al., 2010a). Additionally, these animals had decreased concentrations of IGF-1 which increased throughout rehabilitation and re-alimentation (Richmond et al., 2010a). Furthermore, with a greater caloric intake, the animals had

greater growth rate and body mass coupled with reduced concentrations of GH and IGFBP-2, and greater concentrations of IGF-1 and IGFBP-3 (Richmond et al., 2010a).

Since the hormones of the somatotropic axis in many mammalian species respond to decreased nutritional intake in a similar pattern, the changes that occur in these hormones allow for the impact of nutritional stress on overall growth of animals to be assessed. The main hypothesis for the decline of Steller sea lions in the wild is nutritional stress. This hypothesis may be supported by examining the concentrations of the hormones of the somatotropic axis in these animals. Juveniles are rapidly growing compared with older counter-parts. Thus, juvenile Steller sea lions would be most impacted by nutritional stress and this may be reflected in the changes in the concentrations of the hormones of the somatotropic axis. This would be seen with reduced concentrations of GH and IGFBP-2 and greater concentrations of IGF-1 and IGFBP-3 in these animals.

Conclusion and Thesis

The wild population of Steller sea lions has dramatically decreased in number since the 1970s. This decline, observed only in the genetically distinct Western population, is multifactorial. Many aspects of human interaction and the environment have resulted in decreased quantity and quality of the Steller sea lions prey resources. Ultimately, the overall population numbers have seen a decline as a result of decreased juvenile survival with other age groups having comparable survival rates between populations. Juvenile Steller sea lions are at a disadvantage in times of reduced prey availability as a result of their immature physiology and lack of experience. Growth rate

is strongly connected with survival in many other pinniped species and has implications in the survival of juvenile Steller sea lions.

The somatotropic axis can be used as a tool to assess the growth of juvenile Steller sea lions. Specific developmental changes in the components of the somatotropic axis occur throughout the development of domestic species and will be examined in the Steller sea lion. Various hormone concentrations in juvenile Steller sea lions may be correlated with growth rate and nutritional status. A better understanding the growth of juvenile Steller sea lions will provide information about their survival and ultimately help to better understand the decline of Steller sea lion populations in the wild.

Most studies examining the somatotropic axis in pinnipeds show that the hormones respond similarly in these species as in domestic species. By examining the components of the somatotropic axis that link nutrition, fat metabolism and lean tissue accretion, the impact of decreased nutrient intake on growth rate can be assessed, all of which may have implications for the survival of juvenile Steller sea lions. Our goal is to identify if the components of the somatotropic axis correlate with the growth rate of these developing animals.

We have completed a cross-sectional study of the developmental pattern of the somatotropic axis in Steller sea lions. However, to evaluate the role of the somatotropic axis in regulating growth rate and body composition, individuals must be assessed over time (a longitudinal study) to quantify the relationship of the somatotropic axis to growth rate. Recently, our collaborators at the Alaska Department of Fish & Game have made substantial effort to conduct a longitudinal study of free-ranging juvenile Steller sea lions. With this collaboration we are able to address the primary objectives of this research: 1)

to examine the developmental changes in the somatotropic axis of juvenile Steller sea lions, and 2) to determine if hormone concentrations are correlated with growth rate and body composition. These data will contribute to the understanding of the physiological regulation of growth rate and may contribute to our understanding of the decline of Steller sea lion populations in the wild.

METHODS

Animal Capture and Serum Collection

Between 2005 and 2006, free ranging juvenile Steller Sea lions (n = 24) were captured twice in Prince William Sound, Alaska using the underwater dive capture method (Raum-Suryan et al., 2002). One group was captured at 5 and recaptured at 10 months of age (Group 1) and another was captured at 7 and recaptured at 8 months of age (Group 2). Upon capture, the animals were anesthetized and blood samples (1 mL) were collected from the inter-digital rear flipper vein and body mass, and standard length and axial girth were measured. The age of animals was determined by tooth eruption patterns as outlined by King et al. (2007). Blood samples were collected into heparinized vacutainers and blood was centrifuged for 8 min at 1,380 x *g*. Plasma was then removed and frozen on dry ice before storage at -80°C. The frozen samples were then shipped to the University of Connecticut for hormone analyses.

Hormone Assays

Concentrations of IGF-1 and GH were quantified using radioimmunoassays validated for Steller sea lions (Richmond and Zinn, 2009), whereas concentrations of IGFBP-2 and 3 were quantified using Western ligand blot (Richmond et al., 2010a, 2010b). In this method, serum proteins were separated using SDS-PAGE and then transferred to nitrocellulose membrane (BioRad Laboratories, Hercules, CA). The concentration of serum used per sample was originally determined via serial dilution. The correct concentration was determined to be 0.5 μL per well in the gel. The nitrocellulose was then incubated overnight with the equivalent of 300,000 cpm of

radioactive ^{125}I-IGF-1. The membranes were then washed to remove unbound IGF-1 and the blot was imaged. The membranes were exposed to a multipurpose phosphor screen (Packard Instrument Company, Meriden, CT). The remaining radioactivity bound to the blots was imaged using a Cyclone Storage Phosphor system (Packard Instrument Company) and were then quantified using OptiQuant software.

To quantify the concentrations of IGFBP-2 and -3, digital light units were quantified and values were expressed relative to a bovine standard included on every gel.

Statistical Analyses

Growth rate was determined by subtracting the mass at first capture from the mass at second capture divided by the number of days between captures. Longitudinal measurements of serum GH, IGF-1, and IGFBP-2 and -3, body mass and age were analyzed statistically as repeat measures in a subplot, using a model that accounted for repeated samples from the same experimental unit, with the mixed model analysis of variance procedure (SAS Inst. Inc., Cary, NC). Differences were considered significant if $P < 0.05$ and were considered a trend if $0.05 < P < 0.1$.

RESULTS

Mass of Steller sea lions increased ($P < 0.01$; Figure 1) with age from 69 ± 1.3 kg at 5 mo to 100 ± 2.8 kg at 10 mo (group 1) and 93 ± 5.8 kg at 7 mo to 101 ± 5.7 kg at 8 mo (group 2). Growth rate for Group 1 and Group 2 was 0.21 ± 0.02 kg/day and 0.25 ± 0.08 kg/day respectively. There was no significant difference in growth rate between these different groups ($P = 0.21$).

Concentrations of IGFBP-2 decreased with age from first to second capture (group 1; 37.8 ± 2.5 vs. 36.0 ± 2.5; group 2; 43.7 ± 4.8 vs. 39.8 ± 4.4 AU; $P < 0.01$; Figure 5). Across all animals GH (Figure 2), IGF-I (Figure 3) and IGFBP-3 (Figure 4) averaged 1.6 ± 0.1 ng/mL, 165.7 ± 10.4 ng/mL, 304.0 ± 13.4 AU respectively, but there was no effect ($P > 0.1$) of age on concentrations of these hormones.

Greater concentrations of IGFBP-3 were positively associated with greater growth rate ($P = 0.06$) across all animals. In group 2, the increase in IGF-I concentrations between captures was positively correlated with growth rate ($P < 0.05$)

DISCUSSION

Since the overall population decline of Steller sea lions in the wild is a result of decreased juvenile survival (York, 1994), a better understanding of the growth physiology of free-ranging, juvenile Steller sea lions may have implications for the conservation and recovery of this species.

Steller sea lions are difficult to keep in captivity in sufficient numbers; therefore, it is very difficult to perform a longitudinal study on these marine mammals. Furthermore, their large home range makes repeated capture of individuals very difficult. This study, a recapture of free-ranging, juvenile Steller sea lions, is certainly unique. These data are valuable because they show physiological changes in individuals through development and provide a better understanding of the growth physiology of juvenile Steller sea lions.

Juvenile Steller sea lions range in mass from 64 kg at 5 months of age to 114 kg at 10 months of age in the wild (Richmond et al., 2009). Therefore, individuals in this study were within the normal reported range for these age groups. The lack of difference observed in growth rate in this study reflects other studies of growth in Steller sea lions of these age classes (Winship et al., 2001). During the first year of life, the growth rate of Steller sea lions is around 0.2 to 0.3 kg per day (Winship et al, 2001), which is reflected by the growth rate observed in this study. Differences in growth rate would become more pronounced as these animals get older, as would differences between the growth rates of males and females (Winship et al, 2001).

In most other species, GH is negatively correlated with age and with growth rate (Govoni et al., 2002; Richmond et al., 2010a). Therefore, it was expected that the

concentrations of GH would decrease with age and increased growth rate. However, interestingly, in this experiment, GH concentrations were similar across age groups and there was no change with growth rate. In many young mammals, the concentrations of GH are extremely variable and therefore it is not uncommon for there to be large differences between individuals (Zinn et al., 1989). The data in this experiment do not support this phenomenon. Again, this may be a result of the sampled age classes in that the animals may have been sampled too close in age. The concentrations of these hormones are within the range of population data for free-ranging juveniles for these sampled ages (1.8 ng/mL to 2.9 ng/mL; Richmond et al., 2009). It may be that the concentrations of GH do not begin to decline until further in the development of this species.

The actual concentrations of GH in developing Steller sea lions are similar to arctic foxes (1.25 to 2.1 ng/mL; Fuglei et al., 2004) and rats (2.4 to 2.8 ng/mL; Mustonen et al., 2001). However, the concentrations of GH in Steller sea lions are much greater than those in mink (0.38 to 0.53 ng/mL; Ryökkynen et al., 2003) and raccoon dogs (0.1 to 0.3 ng/mL; Nieminen et al., 2002) in similar juvenile age groups. The greater concentrations of GH in the Steller sea lions, arctic foxes, and rats may indicate a relatively slower growth rate compared with mink and raccoon dogs.

Typically, IGF-1 increases with age and with growth rate in both domestic species and in pinnipeds (Govoni et al., 2002; Richmond et al., 2010a). Therefore, it was expected that the concentrations of IGF-1 would increase with age and growth rate. In this study, the concentrations of IGF-1 were similar across age groups. The observed contradiction in the results is most likely a reflection of the sampled ages. The

concentrations of IGF-1 are similar to population data for these age groups being between 170 and 260 ng/mL (Richmond et al., 2009).

Concentrations of IGF-1 observed in juvenile Steller sea lions are similar to those in other mammals, such as the grey mouse lemur (241 ng/mL; Aujrard et al., 2010), the pig (100 to 200 ng/mL; Harrell et al., 1999), and baboons (215 to 251 ng/mL; Bernstien et al., 2008). However, these concentrations are much greater than those observed in the dog, a carnivorous species (75.2 ng/mL; Eigenmann et al., 1985).

The observed positive correlation between increase in IGF-1 and growth rate is similar to that observed in cattle and pinnipeds (Govoni et al., 2002; Richmond et al., 2010a). As an increased growth rate is associated with an increase in survival in young pinnipeds, these data may give us a tool to assess survival in these animals. A smaller increase in serum concentration of IGF-1 could indicate a smaller growth rate and an animal with a lower chance of survival.

In cattle, the concentrations of IGFBP-2 either show a decrease or remain the same with age (Govoni et al., 2002). Furthermore, the concentrations of IGFBP-2 are negatively correlated with growth rate in domestic species and in pinnipeds (Govoni et al., 2002; Richmond et al., 2010a). Data in the current study showed a decrease in IGFBP-2 concentrations with an increase in age, similar to cattle. Furthermore, these data correspond with population data for free-ranging juveniles between 37 and 41 AU (Richmond et al., 2009).

Current data on the IGFBP are limited; however, some work has been done in swine (Harrell et al., 1999). Harrell et al. (1999) found that during development in the pig, concentrations of IGFBP-2 were between 36 and 66 AU. This is similar to the

concentrations reported in developing Steller sea lions. The changes in IGFBP-2 usually mirror the changes in GH (Govoni et al., 2002; Richmond et al., 2010a). Therefore, since no correlation between GH and growth rate was observed, it is not unexpected that there was no correlation with IGFBP-2 and growth rate. This is most likely a result of the age groups being so close together.

Normally, in domestic and pinniped species, the concentrations of IGFBP-3 correlate with the changes in IGF-1, increasing with age and showing a positive correlation with growth rate (Govoni et al., 2002; Richmond et al., 2009). Data in the current study do not indicate that concentrations of IGFBP-3 are positively correlated with age as observed in these other mammalian species. Again, this is probably a reflection of the age groups sampled. The data in this study reflect population data for free-ranging juveniles being between 303 and 357 AU (Richmond et al., 2009). Since no change in IGF-1 with age was observed, it is not unexpected that the same was true of IGFBP-3.

Harrell et al. (1999) found that during development in the pig, concentrations of IGFBP-3 were between 75 and 285 AU. The concentrations observed in developing Steller sea lions are greater than those observed in the pig.

The positive correlation between IGFBP-3 and growth rate is similar to the pattern observed in domestic species and pinnipeds (Govoni et al., 2002; Richmond et al., 2010a). These data indicate that IGFBP-3 maybe used as a tool to assess growth rate in juvenile Steller sea lions. Since an increased growth rate may indicate an increased chance of survival, it would be beneficial to be able to use IGFBP-3 concentrations to indicate growth rate in these animals.

In many mammalian species, changes in the concentrations of the components of the somatotropic axis reflect changes in growth rate and changes in age. This was not observed with every hormone of the somatotropic axis in this study. However, this is most likely a result of the sampled ages. Overall, these data lead to a better understanding of the growth physiology by showing the changes that occur in components of the somatotropic axis with growth rate in juvenile Steller sea lions. Finally, these data indicate that components of the somatotropic axis may be used as a tool to assess the growth of juvenile Steller sea lions and provides a better understanding of the growth of juvenile Steller sea lions. This may give insight into survival and ultimately help to better understand the decline of Steller sea lion populations in the wild.

FIGURES

Figure 1

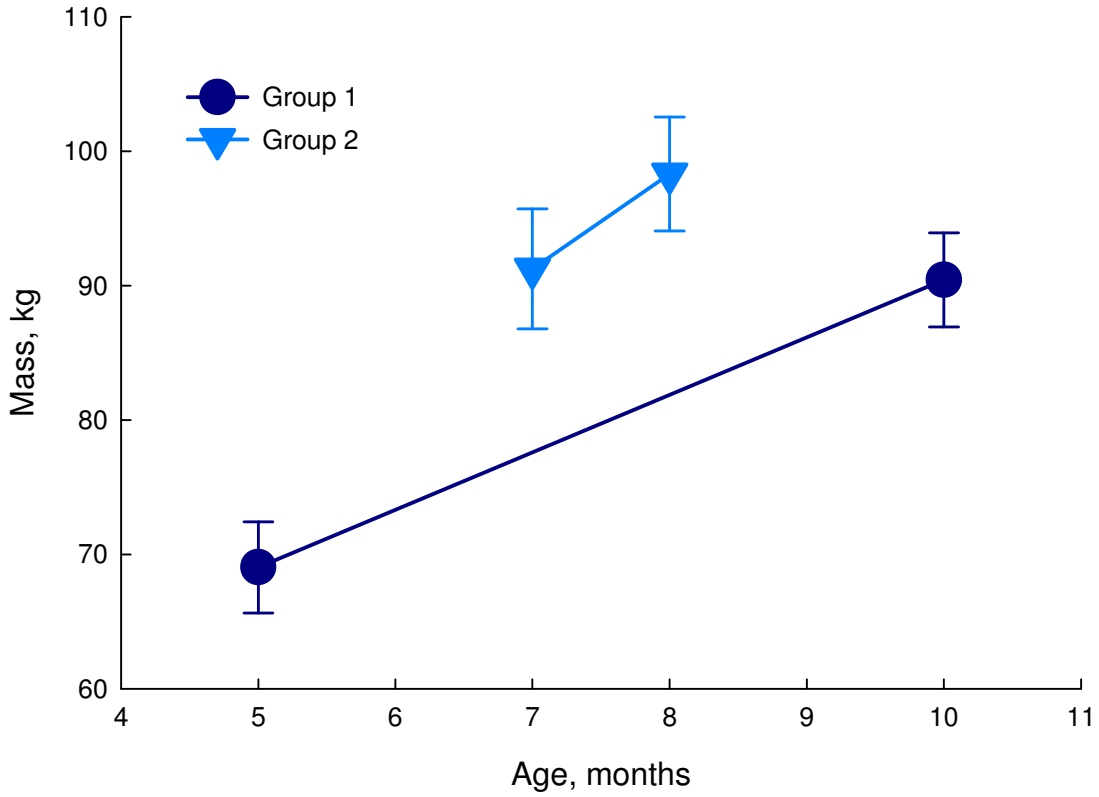

Figure 1: For all ages, there was an increase in mass with increasing age ($P < 0.001$). Growth rate for Group 1 was 0.21 ± 0.2 kg/day and 0.25 ± 0.8 kg/day for Group 2. There was no difference in growth rate between the two age groups ($P = 0.21$).

Figure 2

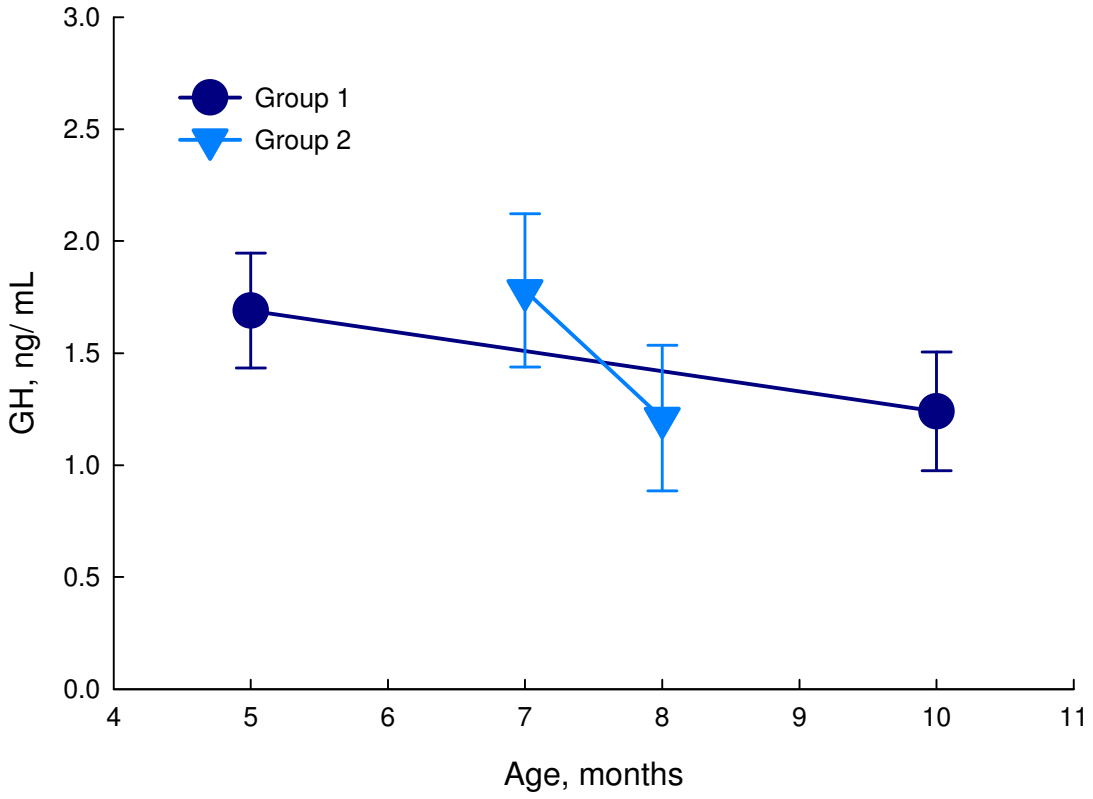

Figure 2: The concentrations of GH were similar among age categories (P = 0.51) and averaged 1.6 ± 0.1 ng/mL.

Figure 3

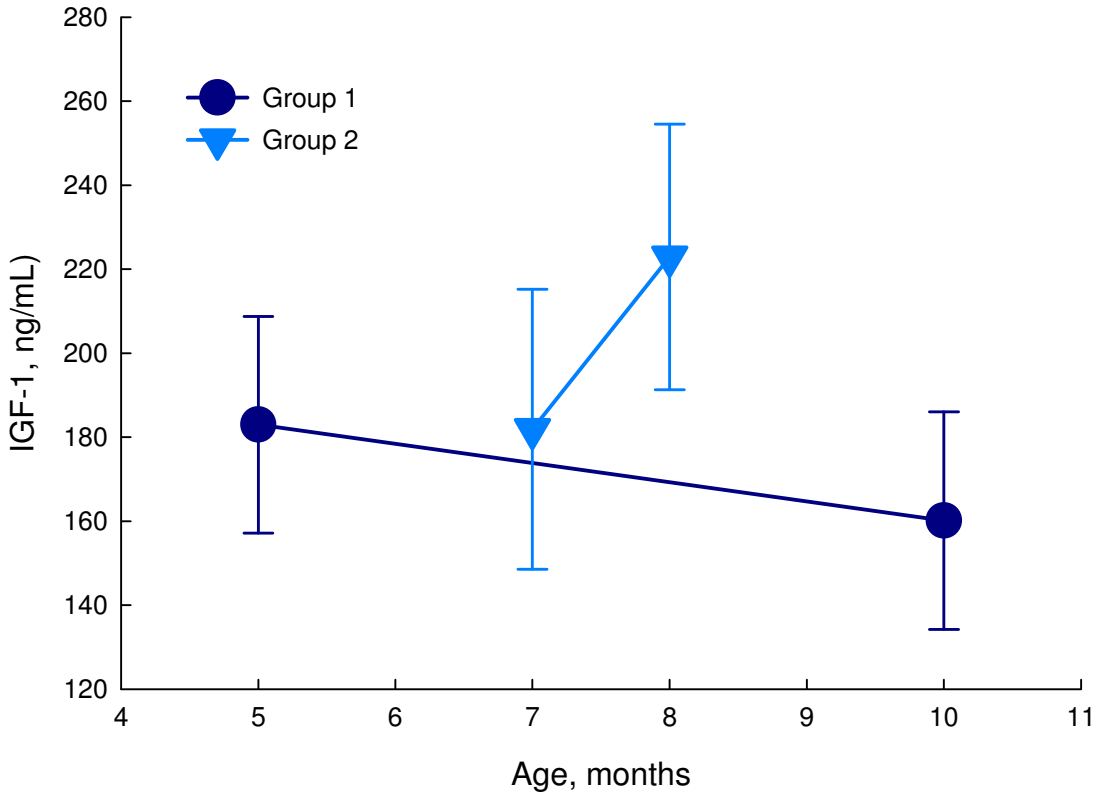

Figure 3: The hormone IGF-1 was similar across age categories (P = 0.46) and averaged 165.7 ± 10.4 ng/mL.

Figure 4

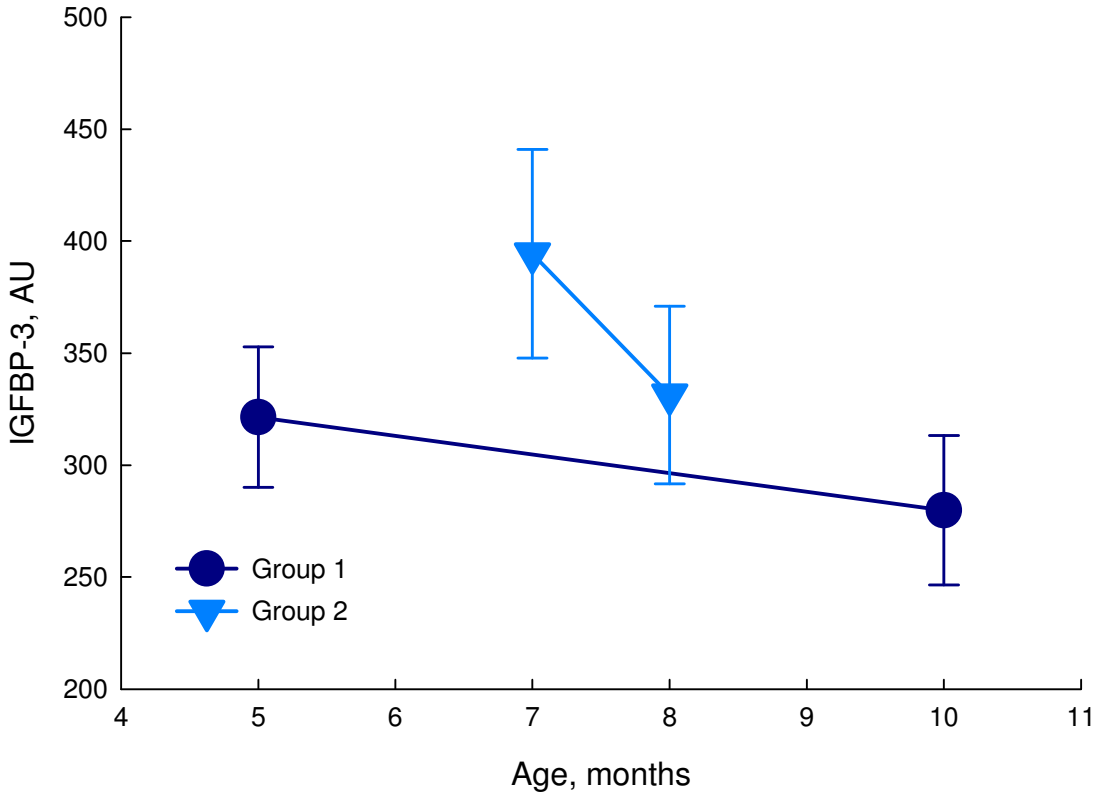

Figure 4: The concentrations of IGFBP-3 were similar across age categories (P = 0.15) and averaged 304.0 ± 13.4 AU.

Figure 5

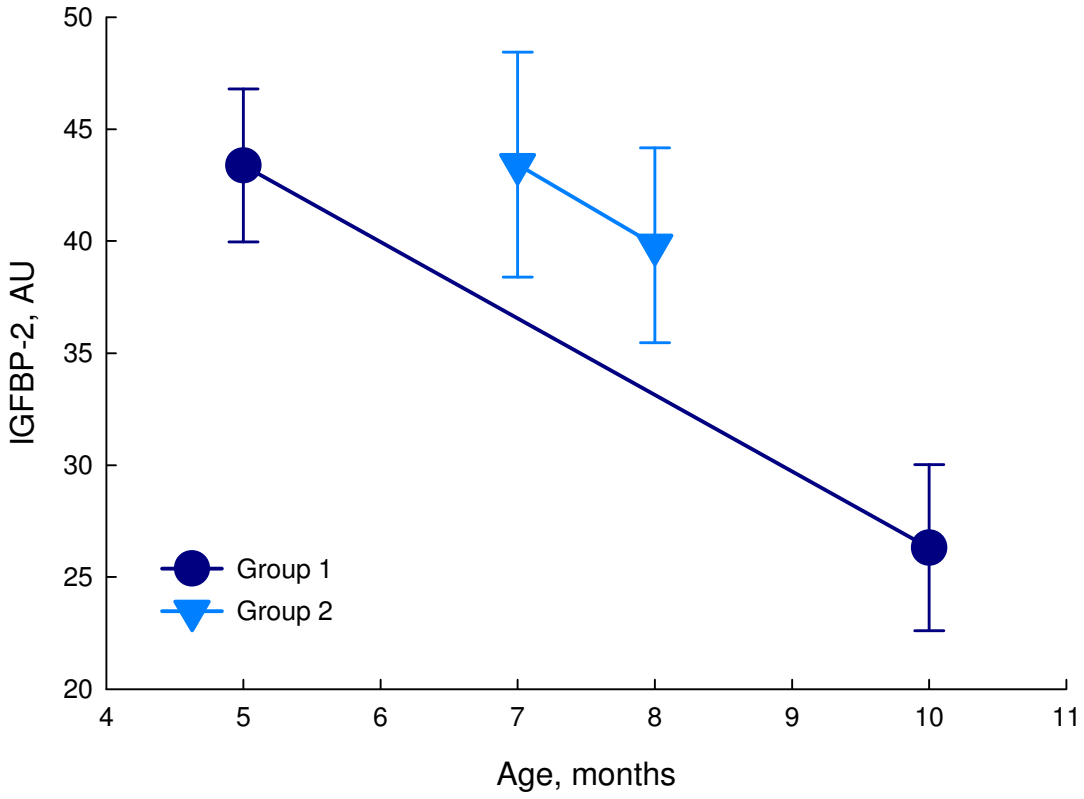

Figure 5: There was a decrease in IGFBP-2 concentrations across age groups (group 1; 37.8 ± 2.5 vs. 36.0 ± 2.5; group 2; 43.7 ± 4.8 vs. 39.8 ± 4.4 AU; $P < 0.01$).

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